



## Original Article



# Effects of *Valeriana officinalis* and Ciprofloxacin on Kidney Histopathology in Rats Pyelonephritis by *Pseudomonas aeruginosa*

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## ABSTRACT

**Introduction:** Urinary tract infection (UTI) is a bacterial infection that affects the urinary tract. The bacterial infection of the upper urinary tract is called nephritis. The purpose of this study was to evaluate the antibacterial effect of *Valeriana officinalis* and Ciprofloxacin on kidney histopathology in rats with pyelonephritis by *Pseudomonas*.

**Materials and Methods:** In this study, 48 male Wistar rats were divided into six groups and each group had two replicates. The first group was considered a control and received 0.1 mg/kg/day of saline daily for a month. The second group received a single injection of 0.5 McFarland of microbial suspension per kilogram of animal into the pelvis of the right kidney of the rats, resulting in pyelonephritis. The third group of rats received 0.9 mg/kg/day methanolic extract of *Valeriana officinalis* intraperitoneally for a month. The fourth group of rats received Ciprofloxacin intraperitoneally for a month at a dose of 0.6 mg/kg/day. Group five contained rats with pyelonephritis that received the antibiotic (Ciprofloxacin) at a dosage of 0.6 mg/kg/day. Group six with pyelonephritis received 0.9 mg/kg/day of *Valeriana officinalis* extract. The inflammation in the cortex, Pelvic, medulla, and tissue sections was studied at the end of the study.

**Results:** The rats that received *Valeriana officinalis* extract improved pelvic and medullary tissue, the site of *Pseudomonas* bacteria, and prevented the destruction of renal cortex tissue. The rats that received Ciprofloxacin had fewer medullary and tissue inflammations.

**Conclusion:** According to the results of this research, the extract not only improved the tissue of the pelvis and medulla, which is where *Pseudomonas* bacteria live, but it also inhibits the degradation of renal cortex tissue. It was proven to diminish medullary inflammation to some extent, but in rats, it exacerbated the loss of renal cortex tissue.

## 1. Introduction

Urinary tract infections (UTIs) are among the most common bacterial infections affecting humans throughout their life span<sup>1</sup>. Pyelonephritis is an infection of the tubular-transsthoracic section of the kidney, in which inflammation involves an involvement of the urethra and renal parenchyma<sup>2</sup>.

Pyelonephritis is a kidney pelvis infection that is degenerative. Acute pyelonephritis is most common in women aged 29-29 years, while the prevalence of urinary tract infections in girls is 11.3% and in boys is 3.6% up to the age of 16. Chemotherapy, diabetes, AIDS, liver illness,

and long-term infections are all factors to consider. Untreated bladder infections, bladder prolapse, pregnancy, enlarged prostate and urinary stones, and urinary fistulas, as well as untreated bladder infections, bladder prolapse, pregnancy, enlarged prostate and urinary stones, and urinary fistulas, can all predispose to this condition. Pyelonephritis can lead to high blood pressure, kidney stones, renal failure, and the need for kidney transplants, as well as dialysis, which can cause miscarriage in pregnant women if left untreated<sup>3</sup>.

The organisms most commonly responsible for

Pyelonephritis are *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, and *Streptococcus faecalis*. *Pseudomonas*, *Serratia*, and *Providencia* species may be responsible for acute complicated pyelonephritis<sup>4,5</sup>. However, there is a paucity of literature in relation to the pathogenesis of UTIs caused by *P. aeruginosa*.

Despite advances in antimicrobial therapy, the mortality and morbidity associated with *P. aeruginosa*-induced UTIs remain significantly high. This unfavorable outcome is due to the inability to develop therapeutic strategies to prevent the disease, which in turn lead to an incomplete understanding of the disease pathogenesis<sup>6,7</sup>.

*Pseudomonas* thrives on various laboratory media, including blood agar and differential media for Enterobacteriaceae growth (such as mechanical). The mucous layer of the urethra is where most germs attach and migrate to the urinary bladder. Bacteria can also travel up the urinary tract and into the kidneys. The inflammation generated by bacteria in the bladder induces significant alterations to transfer the bacteria from the bladder to the ureter<sup>7</sup>.

*Valeriana officinalis* is a perennial plant that contains chemicals from the Fella family, including thymol, tannins and terpenes, pentosan (complex of various sugars), P-Cymene phenols, thymol, carvacrol, and many minerals. Asthma, bronchitis, pertussis, extreme weakness and impairment, stomach pain, kidney diseases, and colds are all treated with thyme<sup>8,9</sup> due to their anti-inflammatory and antibacterial properties.

Since the chemical products available in the market for the treatment of pyelonephritis and the prevention of *Pseudomonas* infection have side effects, and the treatment of damaged kidney tissue in acute pyelonephritis is not easily possible with chemical drugs<sup>8-10</sup>. Furthermore, Given the potential effects of flavonoids and natural antioxidants to prevent and treat pyelonephritis, this study investigated the effects of methanolic extract and glycosides extracted from the *Valeriana officinalis* plant on the pyelonephritis of rats.

## 2. Materials and Methods

### 2.1. Animals

A total of 40 Wistar male rats weighing around 110 g were purchased from Kashan University of Medical Sciences, Kashan, Iran, and kept in the Laboratory Animal Room of the Islamic Azad University, Khorasgan Branch, Isfahan, Iran, for 20 days to adapt to a new environment and gain weight up to 200 g. The laboratory animal room had a temperature of 22°C., a humidity of 30-40%, and a 12-hour cycle of darkness and light. Rat cages were cleaned once a day, and the rats' water and food were checked daily. The rats were removed to the laboratory for weighing and replacement in clean cages. The cages were labeled to indicate that they should not inject or interfere with subsequent tests. The rats received human care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National

Academy of Science<sup>11</sup>.

### 2.2. Microorganisms

The standard strain of *P. aeruginosa* ATCC: 27853 was used in this study. It was maintained in glycerol (50%) and stored at -80°C. Fresh stock was used for each new experiment.

### 2.3. Antibiotic

According to the commercial recommendations, ciprofloxacin (Kimidaro company, Iran) was purchased in powder form and stored in a box away from moisture, light, and temperatures below 30°C.

### 2.4. Preparation of *Valeriana officinalis*

In this phase of the study, 40 g of *Valeriana officinalis* were purchased from the local market in Khorasgan, Iran, and it was finely ground using a mortar. Then, 20 g of powdered material was placed in a soxhlet extraction and was extracted with methanol (250 cc) for 24 hours. The extract was filtered through Whatmann No. 1 filter paper and concentrated using a rotary evaporator below 60°C to generate the extracts of *Valeriana o.* and was finally stored in a desiccator for further studies<sup>12</sup>.

### 2.5. Experimental design

In this experiment, 48 male rats were divided into six groups of eight rats each. The first group was considered as a control and received 0.1 mg/kg/day of saline daily for a month. The second group received a single injection of 0.5 McFarland of microbial suspension per kilogram of animal into the pelvis of the right kidney of the rats, resulting in pyelonephritis. The third group of rats received 0.9 mg/kg/day methanolic extract of *Valeriana officinalis* intraperitoneally for a month. The fourth group of rats received Ciprofloxacin intraperitoneally for a month at a dose of 0.6 mg/kg/day. The fifth group contained rats with pyelonephritis that received the antibiotic (Ciprofloxacin) at a dosage of 0.6 mg/kg/day. The rats with pyelonephritis in the sixth group received 0.9 mg/kg/day of *Valeriana officinalis* extract. The degree of inflammation in the cortex, Pelvic, and medulla sections was studied at the end of the study.

The rats infected with *Pseudomonas* had a larger volume of urine than the control rats. The infected rats were kept in standard conditions and clean cages to prevent other infections. They were not given any medicine for four days until they were fully infected. The medication was administered intraperitoneally for a month after infection<sup>13</sup>.

### 2.6. Histopathological evaluation

Animals were sacrificed under anesthesia, and specimens of all lesions were recorded to investigate possible histopathological changes after the post-mortem

examination. All samples were preserved in neutral buffered 10% formalin. They were dehydrated in ascending concentrations of alcohol, cleared in xylene, embedded in paraffin wax, cut at a thickness of 4  $\mu\text{m}$  (Diapath Company, Itali), stained with hematoxylin and eosin, and examined under a light microscope. Histopathological lesions, including vacuolation, sedimentation, swelling, edema, and occlusion were permeability evaluated<sup>14</sup>.

### 3. Results

The histological findings of the present investigation are shown in [Figure 1](#).

#### 3.1. Pyelonephritis rat

In the cortex, vacuolation and swelling were observed along with atrophy progress. In the medulla, extensive necrosis and congestion were observed, resulting in inflammations with severe infiltration.

#### 3.2. Ciprofloxacin

Atrophy with focal fibrosis was observed in parts of the cortex, moderate necrosis, and atrophy were seen in the medulla.

#### 3.3. *Valeriana officinalis* extract

Mild atrophy and very slight focal inflammation were observed in the normal cortex and in the medulla.

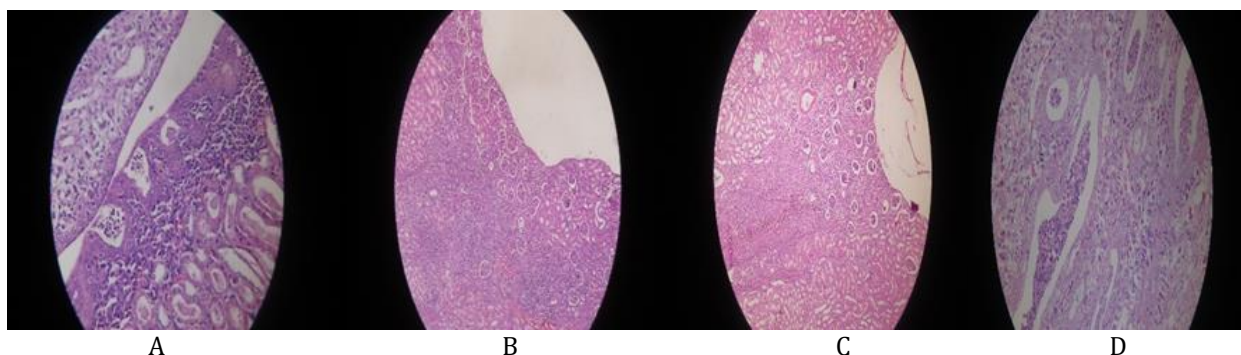
#### 3.4. The result of histological samples

In the cortex, the most inflammation was related to pyelonephritis in rats receiving ciprofloxacin because they received tissue fibrosis in addition to atrophy and vacuolation in pyelonephritis.

The rats with pyelonephritis receiving the extract also showed no signs of inflammation, including vacuolation, swelling, necrosis, fibrosis, or even suffocation, and were normal.

The most common injuries in the pelvis and medulla were observed in the pyelonephritis group with extensive necrosis and concomitant tissue suffocation. However, improvement in rats that received the extract was well seen because they had mild atrophy and very slight focal inflammation.

The pelvic and medullary tissues are the sites of *Pseudomonas* bacteria. The extract could prevent the destruction of renal cortex tissue. However, the rats that received ciprofloxacin indicated a reduced medullary inflammation, which can be related to the bacterial resistant to this antibiotic.



**Figure 1.** Histological findings

A: The kidney of a normal male rat B: The pyelonephritis in an infected male rat D: Pyelonephritis in infected male rats received the extract of *Valeriana officinalis* C: Pyelonephritis in infected male rats received the Ciprofloxacin at a dose of 0.6 mg/kg/day

### 4. Discussion

Urinary tract infection (UTI) is a bacterial infection produced by bacterial pathogens ascending from the bladder to the ureter and infiltrating cells<sup>1</sup>. It can be caused by the destruction of the kidney's pelvic tissue in pyelonephritis or swelling and inflammation of the bladder in cystitis<sup>2</sup>.

The increased use of antibiotics and chemicals with different side effects is now persuading scientists to develop natural substitutes with appropriate effectiveness and protection for humans<sup>15</sup>.

*Valeriana officinalis* continues to be one of the medicinal plants used by today's society for its therapeutic properties and whose biological and pharmacological activities continue to arouse great scientific interest<sup>16</sup>.

*Valeriana officinalis* is claimed to have beneficial roles in the cardiovascular system, known as an anticancer and antimicrobial agent<sup>17</sup>. According to some studies (Rondón et al and Wang et al), essential oils isolated from different *Valeriana* species have significant activity against a wide spectrum of bacterial strains<sup>18, 19</sup>.

In the current study, tissue incisions were sampled from infected rats and treated rats with methanolic extract of *V. officinalis* and Ciprofloxacin. In the cortex, the most common inflammation was in pyelonephritis rats receiving Ciprofloxacin because they had tissue fibrosis as well as atrophy and vacuolation in pyelonephritis. In a study by Miller et al., the administration of an antibiotic at the time or shortly after the induction of experimental renal infection prevented the development of renal lesions without altering the bacteriologic status of the kidney in

the long term<sup>13</sup>.

Eosin and Hematoxylin staining of the lung indicated that the degree of inflammation significantly decreased with no inflammatory nodules after combined ciprofloxacin and ambroxol treatment. In contrast, the level of inflammation remained severe after a single treatment. The pathological lung changes were moderate after ambroxol treatment compared to ciprofloxacin treatment despite no differences in the CFU numbers in the ET tube or lung<sup>22</sup>. The pathological changes in the lungs were also mildest after the combined treatment<sup>22</sup>.

The results of the present experiments showed that the most common damage was severe necrosis with associated tissue blockage in the pyelonephritis group. They were discovered because they showed modest atrophy and small localized inflammation. Tsuchimori et al observed *P. aeruginosa*-induced acute infiltration of neutrophils into the renal pelvis<sup>20</sup>.

Furthermore, the current study indicated that extract-treated pyelonephritis of rats had no signs of inflammation, such as vacuolation, swelling, necrosis, fibrosis, or even suffocation, and were completely normal.

The results of Mittal et al revealed that exaggerated cytokine production during *P. aeruginosa*-induced pyelonephritis could cause tissue damage operative through neutrophil recruitment leading to bacterial persistence in host tissues<sup>21</sup>. Therefore, the data presented have shown that antimicrobial therapy can affect the gross and histopathologic changes in the kidney<sup>21</sup>.

## 5. Conclusion

According to the results of this research, the extract not only improves the status of the pelvis and medulla after inducing infection of *Pseudomonas* bacteria, but it also inhibits the degradation of renal cortex damage. It was proven to diminish medullary inflammation to some extent, but in rats, it exacerbated the loss of renal cortex tissue.

By increasing the limitations of antimicrobial agents and the development of drug resistance, it is necessary to identify, develop, and switch to new natural antimicrobial agents with fewer side effects. The results of the present study can suggest a new treatment material for controlling *P. aeruginosa* infection.

## Declarations

### Competing interests

There is no conflict of interest.

### Authors' contribution

The final manuscript draft was reviewed by all authors, who also gave their approval.

### Funding

None.

## Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

## Ethical considerations

Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by all the authors.

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